Phytosterols and phytostanols lower cholesterol

Coronary heart disease is the leading cause of death in most developed countries today. With an aging and increasingly "at-risk" population, control and preventive measures are an active research area. A high serum cholesterol level has been implicated in numerous studies as a causal factor and guidelines for healthful levels have been formulated by medical societies involved in issuing recommendations and in public education (including The American Heart Association, the American College of Cardiology, and the National Heart, Lung and Blood Institute of the National Institutes of Health). Its importance in long-term control of heart disease is emphasized in a recent analysis of 41 relevant studies: a 10% reduction in serum cholesterol is estimated to result in a 50% reduction of ischemic heart disease at age 50, decreasing to 20% at age 70 (Law *et al.*, 1994). For a large segment of the population, "dietary" cholesterol contributes significantly to serum levels. Hopkins (1992) in an analysis of 27 studies found that the amount of cholesterol in the normal (unrestricted) diet was a stronger predictor of change in plasma cholesterol than added cholesterol, which demonstrated that the rate of cholesterol absorption tends to fall with increasing amounts in the diet. In this article we review past and present research that is leading to a number of new, innovative phytosterol-containing food products that lower blood cholesterol levels in many individuals with hypercholesterolemia.

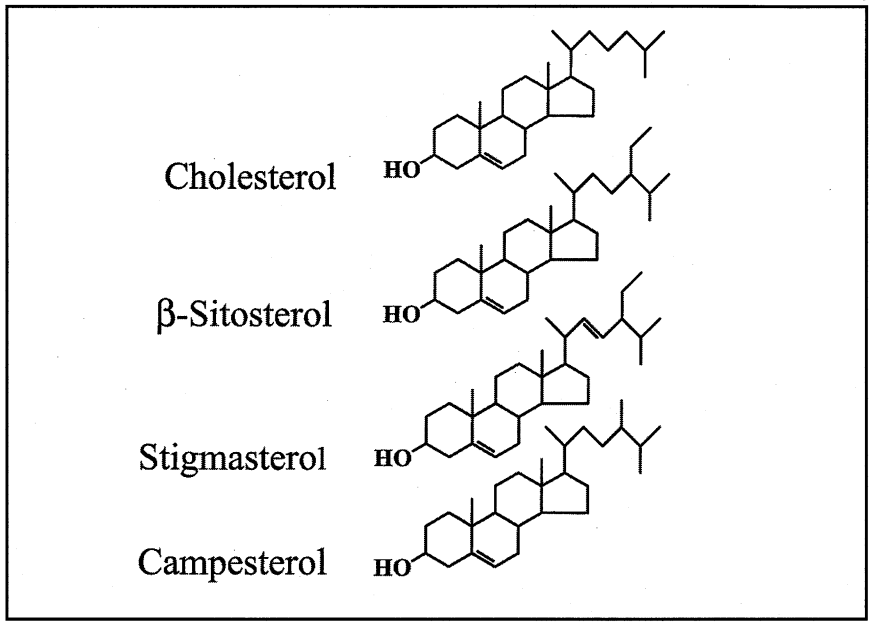
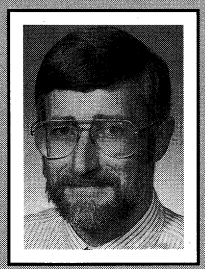
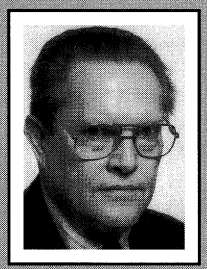


Figure 1. Common animal and plant sterols

Early phytosterol studies and products

There are several pharmacological strategies to lower serum cholesterol but many people would prefer to try to control it without drug treatment, often by restricting dietary cholesterol or by altering the fatty-acid profile of foods eaten. However, the success of this approach has not been high, and researchers have looked for a solution in adding phytosterol-enriched foods or supplements to the diet. The fascinating story of the emergence of phytosterols as cholesterol-lowering compounds is told in Pollak and Kritchevsky's exceptional review (1981), which summarizes hundreds of studies done from the 1950s through the late 1970s. According to Pollak and Kritchevsky, vegetable oils, soy sterols, and β-sitosterol were all suggested as hypocholesterolemic agents at about the same time. Numerous studies were reported in the following decades and some vegetable oils seemed to be able to reduce cholesterol (Pollak and Kritchevsky, 1981). Studies of the components in oils, as well as studies on soy sterols and sitosterol, eventually suggested that the sterol (unsaponifiable) fraction was at least partly responsible for reduction, although fatty acid composition also appeared to be a factor (Pollak and Kritchevsky, 1981). Absorption of


R.A. Moreau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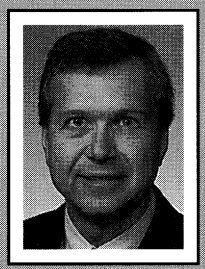
K.B. Hicks

This article on the use of phytosterol and phytostanol products for managing elevated cholesterol levels is by Robert A. Moreau, Eastern Regional Research Center (ERRC), Agricultural Research Service, U.S. Department of Agriculture (USDA), 600 E. Mermaid Lane, Wyndmoor, PA 19038; Robert A. Norton of the USDA National Center for Agricultural Utilization Research; and Kevin B. Hicks of the ERRC.

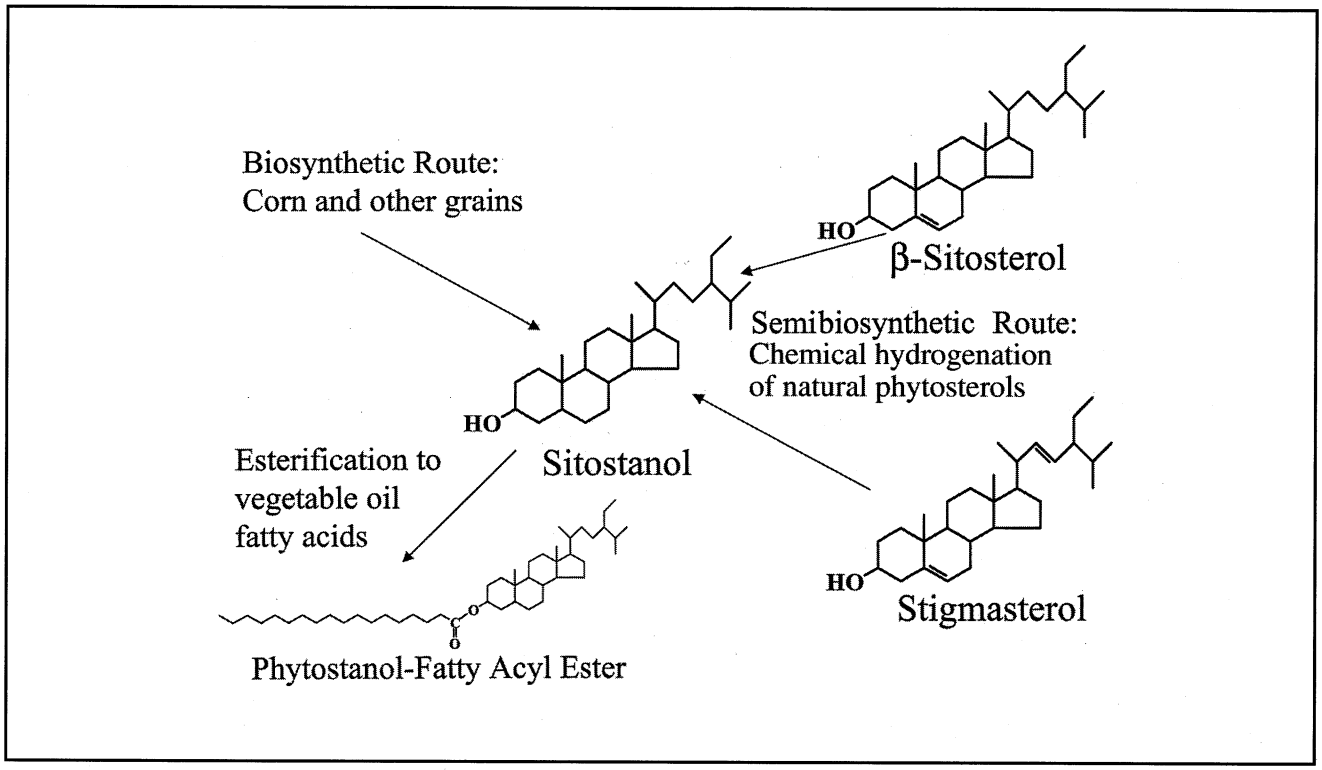

Figure 2. Synthesis of sitostanol and sitostanol-fatty acyl ester

phytosterols in normal individuals is low (5–10%, compared to 45–55% for cholesterol), with the remainder passing through the intestine and removed from the body in the feces (Ling and Jones, 1995). These properties, and the absence of any side effects, made phytosterols attractive candidates for inhibition of cholesterol uptake. Indeed several plant sterol preparations were marketed for this use (Pollak and Kritchevsky, 1981). Free (unesterified) sterols (Figure 1), however, are poorly soluble in fats and oils, and were considered to be expensive and not very effective (Miettinen and Gylling, 1997). In addition, there were objections to the relatively large amounts thought to be required, up to 53 g/d, in some studies (Pollak and Kritchevsky, 1981). The dosage may have been overestimated; 3 g/d of a phytosterol preparation consisting of 90% sitosterol (Figure 1, Cytellin, Eli Lilly Co.) produced the same inhibition of cholesterol absorption as higher levels (Lees *et al.*, 1977). This is the approximate recommended dosage expected to be adopted for the newer products being developed. By the early 1980s use of phytosterol therapy was largely abandoned in favor of drug therapies (Pollak and Kritchevsky, 1981). A recent study of a sitosterol preparation achieved an average reduction of 10% total serum cholesterol and 15% low-density lipoprotein (LDL) cholesterol with a sitosterol supplement of 740 mg/d (Pelletier *et al.*, 1995).

Two developments have caused renewed interest in phytosterols as a treatment for mild to moderate hypercholesterolemia: a series of studies showed that sitostanol, the saturated form of sitosterol, is virtually unabsorbed from the gut and is apparently more effective in preventing cholesterol uptake than the other common plant sterols, including sitosterol, campesterol, and stigmasterol (Miettinen and Gylling, 1997). Secondly, the solubility problem in oils was overcome by esterifying sitostanol with fatty acids (Figure 2). The resultant increase in solubility made possible the incorporation of high levels of sitostanol into a margarine or a mayonnaise (Miettinen and Gylling, 1997). A series of clinical studies by Miettinen and colleagues showed that acceptance of a product of this type was high, compliance good, and LDL cholesterol levels typically decreased by 10–15% in individuals with elevated serum cholesterol (Miettinen and Gylling, 1997).

Sterols in plants

Just as animals do not synthesize plant sterols, plants, with very few exceptions, make negligible amounts of cholesterol. Although plant sterols and some vegetable oils have been shown to be effective in decreasing serum cholesterol, the levels in most foods are not high enough to produce a statistically discernible effect. The sterol content of some common and typical foods and vegetable oils is shown in Table 1. Fruits and vegetables typically contain only small amounts of phytosterols (less than 0.05%, wet weight basis). However nuts and vegetable oils can contain 1% or more of phytosterols. Corn and rice bran oils have the highest levels of sterols of the listed oils. Refining vegetable oils reduces sterols by 20 to 60%

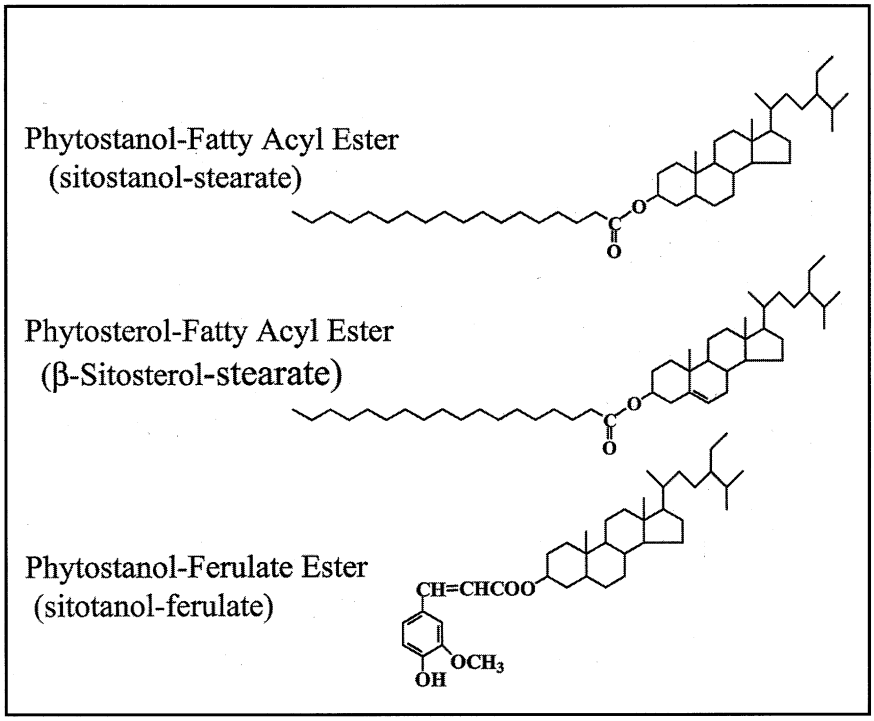

Figure 3. Phytosterol and phytostanol esters

(Weihrauch and Gardner, 1978); hydrogenation decreases sterols by an additional 20 to 40%.

Plant sterols occur naturally in several forms: in the free form (unesterified at the 3-position, see Figure 1), as fatty acid esters (Figure 3), as ferulic or *p*-coumaric acid esters (Figure 3), and as steryl glycosides, which may also be esterified with a fatty acid. Since information is lacking on how glycosides affect cholesterol absorption, they are not considered further here. In most cereals and other higher plants, the principal sterols have a double bond at the 5-position and either a methyl group (campesterol) or an ethyl group (sitosterol) in the side chain at the 24-position. Introducing a second double bond at the 22-position of sitosterol gives stigmasterol (Figure 1). Note that these compounds contain 28 and 29 carbons compared to the 27 found in cholesterol, which tend to make the compounds more hydrophobic than cholesterol. Most of the sterols in plants occur in the free form or as fatty esters; however, the aleurone layer of some cereals (corn, wheat, rye, and triticale) have a high percentage of saturated phytosterols (i.e., sitostanol and campestanol) which are esterified to ferulic acid (Figure 3) and, in corn to a much lesser extent, *p*-coumaric acid (Seitz, 1989). ["Stanols" is used to refer to saturated sterols as a group. Rice bran differs from most other cereals in having mostly 30- and 31-carbon sterols (triterpenoids) esterified to ferulic acid and small amounts of typical Δ-5 sterols.]

Mechanism of cholesterol absorption inhibition

The mechanism by which phytosterols reduce cholesterol has been studied. Before discussing this, a brief overview of the fate of dietary sterols will be presented. Fats are converted to a coarse emulsion in the stomach and released into the duodenum where they are mixed with bile and pancreatic juice. Triglycerides, sterol esters, and phospholipids are cleaved by pancreatic lipases and form mixed micellar particles (Wilson and Rudel, 1994), although oil and other phases are also present. The products of lipid digestion include fatty acids, glycerides, lysophosphatidylcholine, and free cholesterol. The micelles diffuse through a static water layer and a layer of mucoprotein to the brush border membrane (BBM) of intestinal epithelial cells (enterocytes) lining the interior of the proximal small intestine. Cholesterol is thought to be absorbed in the monomolecular form from the intermicellar aqueous phase, which is rapidly replenished from the micelles with the micellar phase functioning as a reservoir (Wilson and Rudel, 1994; Thomson *et al.*, 1989). Cholesterol and other micellar components are then passively absorbed by interaction with scavenger receptor BI (SR-BI) protein located in the BBM. SR-BI facilitates the uptake of sterols, sterol esters, and other lipids (Hauser *et al.*, 1998). After esterification to fatty acids, cholesterol is packaged into chylomicrons and, to a lesser extent, very low density lipoproteins (VLDL) and secreted into lymphatics for transport to the liver by the bloodstream. Many factors in the gut affect cholesterol uptake, including cholesterol concentration; pH; type and concentration of bile acids; the ratio of lyso- and phosphatidylcholine; and types of fatty acids present as well as other sterols (Thomson *et al.*, 1989).

Studies indicate that phytosterols exclude cholesterol from the mixed micellar phase (Ling and Jones, 1995). The presence of the methyl group in the side chain of campesterol and the ethyl group in sistosterol makes the phytosterols more hydrophobic than cholesterol and increases their affinity for mixed micelles (Ling and Jones,1995). Sitostanol has been shown to be more effective than other phytosterols in preventing cholesterol absorption (Miettinen and Gylling, 1997) and has the added advantage of not being absorbed, which may allay some concerns over side effects from higher serum levels of phytosterols (Ling and Jones, 1995).

Phytosterols appear to vary in their absorbability. Campesterol is absorbed more than sitosterol, but campestanol, unlike sitostanol, may be absorbed to a greater extent than other plant sterols (Heinemann *et al.*, 1993). However, recent results suggest that if sitostanol is included in the diet, campestanol is not absorbed (Hallikainen and Uusitupa, 1999) and the absorption of other plant sterols may be decreased as well (Miettinen and Gylling, 1997).

Table 1
Sterol composition of various raw foods and refined oils[a]

Food	Cholesterol (mg/100 g)	Phytosterols (mg/100 g)
Skim milk	1.8	
Whole milk	13.6	
Cottage cheese	15	
Cream cheese	108	
Butter	219	
Margarine, soybean[b]		146
Margarine, corn[b]		570
Egg, whole	425	
Turkey, light meat	60	
Pork, ground	72	
Chicken, whole	75	
Beef, ground, lean	75	
Liver, beef	354	
Tuna	38	
Cod	73	
Lobster	95	
Shrimp	152	
Potatoes		5
Tomatoes		7
Pears		8
Lettuce, iceberg		10
Carrots		12
Apples		12
Onions		15
Bananas		16
Figs		31
Garbanzo beans		35
Kidney beans		127
Soybeans, mature		161
Pecans		108
Almonds		143
Cashew nuts		158
Peanuts		220
Sesame seeds		714
Peanut oil		207
Olive oil		221
Soybean oil		250
Cottonseed oil		324
Safflower oil		444
Sesame oil		865
Corn oil		968
Rice bran oil		1190

[a] From U.S. Department of Agriculture food composition website.
[b] Contains 15.7% water.

A typical North American adult consumes 300–500 mg/d of cholesterol; bile provides an additional 800–1,200 mg/d to the intestinal pool and an estimated 250–400 mg/d comes from turnover of the intestinal epithelium (Wilson and Rudel, 1994). During passage through the intestine, cholesterol in the bile micelles equilibrates with dietary cholesterol (Wilson and Rudel, 1994). Thus the total pool of cholesterol available for absorption in the intestine is considerably larger than the amount in the diet. The effective daily dosage of phytosterols (or phytostanols) is estimated to be about four to six times the level of dietary cholesterol for inhibition of cholesterol absorption, and this is the reason taking phytosterols with meals is recommended. Sitostanol reduces cholesterol absorption by 45–60% (Meittenin and Gylling, 1997). However, reduction in dietary cholesterol does not result in an equivalent reduction in serum cholesterol due to compensatory, homeostatic biosynthesis. The net result is that dietary restriction or inhibition of cholesterol uptake by phytosterols have generally been able to reduce LDL cholesterol by 7–15% in humans, but do not show an effect on levels of high-density lipoprotein (HDL) cholesterol, VLDL cholesterol, or serum triglyceride (Meittenin and Gylling, 1997).

Phytostanol ester products: chemistry and efficacy

A series of papers from Finland have demonstrated the clinical cholesterol-lowering efficacy of phytostanol ester margarine (90% sitostanol, 6% campestanol). The phytostanols used in these studies were obtained by hydrogenating tall oil (an abundant byproduct of wood pulping) phytosterols and then esterifying them to rapeseed oil fatty acids (Figure 2). Miettinen *et al.* reported in 1995 a study where 100 mildly hypercholesterolemic persons were put on a diet that contained sitostanol ester margarine (1.8–2.6 g sitostanol per day). After four weeks, the levels of total and LDL cholesterol were lowered by 10% and 14%, respectively. A sitostanol ester margarine, Benecol, has been marketed in Finland for several years by Raisio Co. A U.S. patent for producing sitostanol ester margarine was issued to Raisio (Miettinen *et al.*, 1996), and licensed to McNeil (a division of Johnson and Johnson) in 1998. McNeil's initial proposal to market Benecol as a dietary supplement was opposed by the U.S. Food and Drug Administration. The two have since been negotiating Benecol's use as a food ingredient. McNeil has reported plans to start marketing Benecol in the United States during 1999. In a recent clinical study (Hallikainen and Uusitupa, 1999) the efficacy of a wood-derived stanol ester margarine (Benecol) and of a vegetable oil-derived stanol ester margarine were examined; they

were found to be equally effective at lowering serum cholesterol.

Phytosterol ester products: chemistry and efficacy

Westrate and Meijer (1998) recently published a clinical study designed to compare the cholesterol-lowering efficacy of soy phytosterol–fatty acyl ester margarine with phytostanol fatty acyl ester margarine (Benecol). In this study, 100 normal or mildly hypercholesterolemic individuals were placed on diets that included margarine containing either soy phytosterol–fatty acyl esters (3.2 g/d) or phytostanol–fatty acyl esters (2.7 g/d) for 3.5 weeks.

The levels of total cholesterol were reduced 8.3% with the soy phytosterol margarine and 7.3% with the phytostanol margarine. Similarly, the levels of LDL cholesterol were reduced 13% with either phytosterol margarine or the phytostanol margarine. Two other margarines (one containing rice bran oil phytosterols and another containing shea nut phytosterols) had no significant effect on total or LDL cholesterol values. The results of this clinical study suggested, for the first time, that the less-expensive phytosterol–fatty acyl esters (fed at a 19% higher dosage) were as effective as phytostanol fatty acyl esters. The controversial nature of these results (Westrate and Meijer, 1998) and the potential cost savings which could be realized by not having to hydrogenate phytosterols to phytostanols (Figure 2) were pointed out in a recent review (Jones and Ntanios, 1998).

Unilever has said it plans to market phytosterol-fatty acyl ester margarine and salad dressing in the United States (under its Lipton division with the brand name of Take Control) and in other countries.

Free (nonesterified) phytosterol products: chemistry and efficacy

Several companies have marketed free phytosterol-containing dietary supplements. Although most of the free phytosterol products have been obtained from vegetable oils, Forbes-Meditech has recently developed a process to obtain a free phytosterol-rich fraction from tall oil. Tall oil contains phytosterols and phytostanols (at a ratio of about four to one). Forbes-Meditech has announced two types of products that contain micronized (reduced to no larger than microscopic size) tall oil phytosterols: "Phytrol," a proprietary nutraceutical, and "CardioRex," a proprietary pharmaceutical product. The efficacy of "CardioRex" was reported in a recently published animal feeding study (Moghadasian *et al.*, 1999).

In an April 19, 1999, news release, Forbes-Meditech announced that it has granted Novartis Consumer Health AG a license "to exclusive worldwide rights to use or sub-license Phytrol for use in functional foods, dietary supplements and over-the-counter products." In a recent clinical study (Jones *et al.*, 1998), 22 mildly hypercholesterolemic persons were fed a diet enriched in micronized tall oil phytosterols (21.2 mg phytosterols/d/kg body weight = ~1.5 g phytosterols/d/160 lb person). During the 10-day study, the tall oil phytosterol diet caused a 6% reduction in total cholesterol and a 10% reduction in LDL cholesterol.

Corn fiber oil

Commercial corn oil is obtained by extracting the germ portion of the corn kernel and should more precisely be called "corn germ oil." Whereas commercial corn oil contains >98% triacylglcyerols, we recently discovered (Moreau *et al.*, 1996) a second type of corn oil, corn fiber oil, which contains high levels of both phytosterols (1–2% free phytosterols and 3–9% esterified as fatty acyl esters) and phytostanols (3–7% free, with ~70% esterified as ferulate esters of sitostanol and campesterol, Figure 3), with the remainder being triacylglycerols (approximately 80–90 wt%). The unusually high level of sitostanol in corn fiber oil makes it the richest natural source of this rare phytostanol yet reported. Corn fiber oil is obtained by extracting corn fiber, a pericarp-enriched low-value by-product of the wet-milling of corn for starch, fuel ethanol, and corn syrups. We developed a process to obtain corn fiber oil and have patented its composition, preparation and use as a cholesterol-lowering natural product. (Moreau *et al.*, 1998). Monsanto licensed this patent in 1997. Corn fiber oil was shown to reduce total and LDL cholesterol in hamsters (Moreau *et al.*, 1998), but clinical studies have not yet been reported.

Interestingly, corn fiber oil is the only product that contains a natural mixture of three of the important classes of phytosterols (free phytosterols, phytosterol fatty acyl esters, and phytostanol esters). As mentioned above, the phytostanols in corn fiber oil are esterified primarily with ferulic acid, rather than fatty acids as they are in Benecol. The strong antioxidant activity of ferulic acid may give corn fiber oil additional healthful properties.

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